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Original Research Article

Comparative Analysis of Dengue Infection Patterns, Severity, and Clinical Outcomes in a Tertiary Care Teaching Hospital

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Abstract:

Introduction: A seasonal acute febrile arbo-viral disease, dengue fever can range from an asymptomatic infection to severe dengue hemorrhagic fever and dengue shock syndrome. Outbreaks of dengue hemorrhagic fever are frequently reported in seven countries in South-East Asia, including India.

Objectives: To identify dengue seropositive patients by NS1 antigen testing and anti-dengue IgM antibody detection by ELISA and correlate the changes in epidemiology.

Materials and Method: Retrospective study done from January to December 2024 with blood samples tested from clinically suspected cases of dengue virus infection.

Results: 3839 samples in all were examined. MAC ELISA recorded 488 (12.71%) positives, while NS1 ELISA identified 196 (5.11%) cases. Reports of cases peaked between June and September. With a general male predominance, the incidence was high in the paediatric age group, five (3.12%) in 2024, and one (0.65%) in 2023.

Conclusion: Prevalence of dengue seropositive cases was 34.20% in 2024, 19.07% and 14.89% in 2024 respectively indicating a relative decline in dengue infection which may be attributable to the increase in awareness and preventive measures taken among the people and health services.

Keywords: Dengue fever, Dengue haemorrhagic fever, NS1 Ag, IgM, ELISA.

Introduction

In India, dengue is one of the most significant arboviral infections. The single positive stranded enclosed RNA virus that causes dengue fever belongs to the Flaviviridae family, specifically the genus Flavivirus. The Dengue virus has four distinct serotypes: DENV-1, DENV-2, DENV-3, and DENV-4. These are further divided into subtypes according to their unique genotypes. [1] In October 2013, a new serotype (DENV-5) that follows the sylvatic cycle was discovered in Malaysia's Sarawak state through genetic sequence research.[2]

Although India has isolated the DENV-1, DENV-2, DENV-3, and DENV4 serotypes, the bulk of illnesses are caused by DEN V1 and DEN-2. Female mosquitoes of the species Aedes aegypti and, to a lesser extent, Aedes are the primary vectors of dengue virus transmission Albopictus. It attacks during the day.

Dengue fever is the most significant tropical infectious illness after malaria, with an estimated 100 million cases, 500,000 cases of dengue hemorrhagic fever, and 25,000 fatalities each year in 1998.[3] There is a chance of perinatal transmission, which could make the newborn more sick. Additionally, organ transplants, needlestick injuries, and blood transfusions can all spread dengue. A significant public health concern in terms of mortality and morbidity is dengue virus infection.[4]

In many regions of India, dengue is endemic.[5] The first clinical dengue-like sickness pandemic in India was documented in Chennai in 1780, and the first epidemic with virological proof took place in Kolkata and along India's Eastern Coast in 1963–1964.[6] The first report of infant DHF/DSS was made in 1970.Severe dengue has a relatively high case fatality rate in Asian nations.

Dengue fever, also referred to as break-bone fever, is a brief, self-limiting sickness. After an infected mosquito bite, the incubation period lasts anywhere from three to seven days. Most infections result in the development of typical dengue fever, but other infections remain asymptomatic. High temperature, headache, nausea, vomiting, retroorbital discomfort, rash, myalgia, arthralgia, joint and muscle pain, and bleeding symptoms are some of the symptoms. It usually takes two to seven days to recover. From

mild, self-limiting dengue fever to severe forms of the disease, including dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), the condition can vary widely. When a subsequent dengue infection occurs, DHF is commonly observed. However, it can also happen to babies. during an initial infection brought on by dengue antibodies acquired by the mother.[7] For four to five days following the start of the disease, the virus can be found in serum, plasma, circulating blood cells, and other organs. One serotype infection results in a high titre neutralizing antibody, and recovery from infection offers only partial protection against infection with other serotypes but lifetime immunity against reinfection by the same serotype. Viral culture, viral RNA detection by RT-PCR, and serological tests such NS1 Ag detection and IgM and IgG capture ELISA are the main diagnostic techniques. Since RT-PCR is more sensitive and specific, it may be used to detect serotypes and quantify viral loads. Inoculation into mosquito cell lines C6/36 and AP 61 is the primary method of virus isolation utilized for research purposes. A highly conserved non-structural protein for all serotypes of the dengue virus, NS1 Ag is released from infected cells and enters the bloodstream early. The NS1 antigen triggers a robust humoral immune response. From the first day of fever, the NS1 antigen can be found and stays positive for up to eighteen days. The host humoral immune response and the degree of viremia determine the sensitivity of the NS1 antigen-capture ELISA. The NS1 Ag assay is a helpful early diagnostic indicator for dengue fever that enables quick identification on The NS1 antigen can be found as early as the first day of a fever and stays positive for up to eighteen days. The degree of viremia and the humoral immune response of the host determine the sensitivity of the NS1 antigen-capture ELISA. The NS1 Ag assay is a helpful early diagnostic marker for dengue fever that enables prompt diagnosis before antibodies show up on the first day of fever. Without revealing the serotype, a positive NS1 test result verifies dengue virus infection.

IgM appears first after 5 days of fever and disappears within 90 days 8and the antibody levels are consistent with acute-phase infection. Cross reactivity with several other flaviviruses like Zika virus, West Nile virus, and St. Louis encephalitis virus may lead to false positive results with IgM ELISA. MAC ELISA (IgM antibody capture ELISA) is the recommended serological test in India. The dengue IgG detectable at low titre in 14-21 days and then slowly increases, and persist for months or years which often reactive with many flaviviruses. Of these three different tests, NS1 antigen detection had the highest sensitivity in the early stages while IgM detection was more sensitive in the later part of the illness. Positive dengue IgM and IgG tests for dengue antibodies in an initial blood sample means that the person became infected within recent weeks. According to WHO dengue-specific laboratory tests are often not required for acute management of cases but should be performed to confirm the diagnosis. Dengue fever is usually a self-limited illness. Appropriate clinical management can save the lives of patients with DHF and DSS and mortality can be reduced to less than 1%.[9] Prevention of dengue virus transmission entirely depends on effective vector control measures or interruption of human–vector contact. Although there is no specific treatment available for dengue, accurate and timely diagnosis has an important role in individual case management and in planning and implementing control strategies.

Aim: To investigate and compare the patterns of dengue infection, including demographic and seasonal distribution, assess the severity of the disease, and analyze clinical outcomes among patients presenting to a tertiary care hospital.

Materials and Methods

Period of study: One year, January 2024 to December 2024. Consent from individual patients was obtained prior to blood collection. Inclusion Criteria The patients with clinical feature suggestive of dengue fever and DSS/DHF cases irrespective of their age and sex were included in this study. A total of 1194 serum samples (males-678, females516) from suspected dengue fever cases were collected from patients with acute febrile illness, headache, myalgia, arthralgia, rashes and bleeding tendencies under aseptic precautions. Study group included both inpatients and outpatients belonging to age group 3 to 87 years.

A single blood sample (approximately 2-3 ml) was collected from clinically suspected patients by venepuncture according to the Clinical and Laboratory Standards Institute (CLSI) standard protocol and allowed to clot at room temperature.

Samples were then centrifuged, serum separated, and subjected for NS1 antigen assay by QUALISA Dengue NS1 (QUALPRO DIAGNOSTICS), dengue-specific IgM antibodies by using IgM antibody capture enzyme-linked immunosorbent assay (NIV DEN IgM Capture ELISA kit from National institute of virology). The tests were done according to the manufacturers guidelines and results were recorded based on OD value. Few samples were tested for dengue IgG. Data were analysed

Statistical Analysis: Data was entered into Microsoft Excel and analysed using PASW (Predictive Analytics Software) Version 18.0. Descriptive statistics such as percentages were used. Inferential statistics such as Chi- square test were used to find out association between variables.

Differences with p- value <0.05 were considered statistically significant.

Results

Out of 1194 samples tested, 258 samples (21.6 %) gave a positive serological test for acute dengue viral infection [Figure 1]. Out of 258 seropositive cases, NS1 antigen was found to be reactive in 116 (9.71%) and IgM in 142 (11.89%), samples [Table 1] Seropositivity more during the month of July, (25.88% for NSI antigen and 15.85% for IgM antibody) [Table 2].

Both males and females were affected almost equally (male 57.36%, female 46.64%) with a male to female ratio 1.3:1. [Table 3] The association between gender and dengue seropositivity was not found to be statistically significant [Table 4]. Seasonal variation was observed. Only few cases were present during the month of January to May and positivity peak noted between the months of June to September [Figure 2&3]. The most affected age group was 20-29 years followed by 30-39 years as 34.4% and 32.5% [Figure 4]. The association between age and dengue sero-positivity was found to be statistically significant. It was found that dengue sero-positivity was found to be more among those aged 50years or younger as compared to those older than 50years. There is 2 times more risk of contracting dengue fever in those aged ≤ 50 years. [Table5] It is noticed that infection was prevalent throughout the year with seasonal spurts in monsoon and post monsoon. Highest percentage of suspects and cases were seen during the months June-July (2.68%and 8.79%). Dengue sero-positivity was found to be more during Monsoon Season (27.2%) as compared to other seasons and the association was found to be statistically significant (p- value <0.001) [Table 6]

Table 1: Age wise distribution of cases

Age in years	Total cases	NS1	IgM
0-9	12	2	0(16.6%)
10-19	56	3	2((8.92%)
20-29	180	30	32(34.4%)
30-39	240	32	46(32.5%))
40-49	364	24	30 (14.83%)
50 and above	342	25	32 (16.7%)
Total	1194	116	142(21.6%)

Table 2: Month wise distribution of cases

Month	Total samples tested for Dengue NSI and IgM	Samples positive for NS1	Samples positive for Dengue IgM	Total NS1+IgM
January	24	3	1	4
February	33	0	3	3
March	25	0	2	2
April	13	1	2	3
May	12	4	6	10
June	57	19	13	32
July	255	66	39	105
August	84	13	20	33
September	357	19	16	35
October	133	7	11	18
November	117	6	3	9
December	84	4	0	4
Total No	1194	142	116	258

Table 3: Distribution of Dengue by gender

Gender	Total cases	NS1 positive	IgM positive	Percentage
Male	678	78	70	57.36%
Female	516	64	46	42.64%
Total cases	1194	142	116	100%

Table 4: Gender distribution and association with Dengue seropositivity

Gender	Dengue Positive	Negative	Total	p-Value	Odd's Ratio (95% CI)
Male	148 (21.8)	530 (78.2)	678 (100)	0.88	1.03 (0.78-1.362)
Female	110 (21.3)	406 (78.7)	516 (100)		
Total	258 (21.6)	936 (78.4)	1194 (100)		

Table 5: Association between age and Dengue seropositivity

Age	Dengue Positive	Dengue Negative	Total	p-Value	Odd's Ratio (95% CI)
≤ 50YRS	201 (25.3)	594 (74.7)	795 (100)	0.00001863 (<0.001)	2.03 (1.47- 2.804)
> 50 YRS	57 (14.3)	342 (85.7)	399 (100)		
Total	258 (21.6)	936 (78.4)	1194 (100)		

Table 6: Association of Dengue seropositivity and seasonality

Season	Dengue Positive	Dengue Negative	Total	P-Value	Odd's Ratio (95% CI)
Monsoon Season	205(27.2)	548(72.8)	753 (100)	<0.0000001	2.74 (1.97-3.80)
Other Season	53(12.1)	388(87.9)	441 (100)		
Total	258 (21.6)	936 (78.4)	1194 (100)		

Discussion

Dengue virus is a mosquito-borne enveloped single-stranded RNA virus which belongs to Flaviviridae family, genus Flavivirus and is transmitted by mosquitoes especially Aedes egypti. Dengue fever is also known as break bone fever because of severe muscle, joint and bone pains. Dengue is a Spanish homonym for the "Swahili ki denga pepo" which describes a sudden, cramp like seizure caused by an evil spirit. Five serotypes of the virus have been found, all of which can cause disease. People of all age groups who are exposed to infected mosquitoes are victims of dengue fever.

According to World Health Organization (WHO), dengue fever is a mosquito-borne viral infection, found in tropical and sub-tropical climates worldwide, mostly in urban and semi-urban areas**.** Based on severity of infection there are two stages of dengue, dengue with or without warning signs and severe dengue (2009 WHO classification). The first recorded epidemic of dengue was reported in the late 18th century which affected Asia, Africa, and North America. Dengue haemorrhagic fever (DHF) a serious complication of dengue fever, and a life- threatening emergency is characterised by increased vascular permeability and abnormal blood clotting mechanisms. Dengue shock syndrome can occur after 2-7 days of dengue haemorrhagic fever.

Virus isolation, nucleic acid antigen or antibody detection can be used to diagnose the acute dengue infection. In acute phase of infection, serology is the method of choice for diagnosis. Of the NS1Ag, IgM and IgG tests used to diagnose dengue, NS1 antigen detection had the highest sensitivity in the early stages of illness while IgM detection was more sensitive in the later stage.

At present, there is no specific treatment available for dengue infection. Early diagnosis has a crucial role in management and implementation of control strategies. The first dengue vaccine, Dengvaxia (CYD-TDV) by Sanofi Pasteur, was first registered in Mexico in December 2015 is available in some countries. Other methods of prevention include reducing mosquito habitat and limiting exposure to mosquito bites. NS1 Ag and IgM detection by ELISA are potentially useful tests during acute dengue infection. In the present study, out of 1194 samples tested, 258 (21.6%) were serologically positive for NS1 Ag,142 (11.89%) and IgM antibody116 (9.71%) which suggests primary infection. Overall prevalence of dengue during the study period was 21.6%. In a study by Ashwini et al showed the highest positive detection rate of NS1 and IgM (97.8%).[10] In this study, a total of 94 cases were confirmed out of 112 clinically suspected cases with NS1 antigen positivity rate of 80.9 % and IgM 47.9%.

In the present study, out of 1194 suspected patients 116(9.71%) samples were IgM positive which indicated recent dengue infection. In a study by Sailaja et al, conducted in Andhra Pradesh, out of 108 cases, 33 (30%) were positive for IgM antibody between fifth to tenth day of fever.[11] In a study conducted by Neralwar A et al,[12] in Raipur, out of 1637 samples tested for Dengue infections, 161(29.92%) samples were NS1 positive, 83 15.42%) were both NS1 +IgM Ab positive and 294 (54.64%) were IgM Ab positive.

In this study out of 1194 samples 142 (11.89%) were positive for NS1 Ag. In a study by Fauziah MdKassim et al,[13] out of the 208 sera tested, 69.2% (144/208) sera were positive for dengue virus infection. Of these (32.2%) samples (67/208) were found positive for dengue NS1 antigen only. Datta et. al had reported NS1Ag positivity which varied from 71.42% to 28.4% in acute and early convalescent sera, conversely IgM detection rate was 93.61% and 6.38% in early convalescent and acute phase sere.[14] In a study conducted in Angul district of Odisha by Dharitri Mahapatra et al out of the 1020 blood samples screened, 513 (50.2%) were positive for dengue NS1 Ag.[15]

The NS1 antigen and IgM antibody ELISA positivity rates are 25.59% and 17.3% respectively in study by Rajesh Kumar Varma et al conducted in Western Uttar Pradesh.[16] In another study conducted in Nepal, anti-dengue IgM was found in 8.5% of patients (50/590 cases).[17]

The IgG antibody level is very crucial in secondary infection of dengue virus. In this study only 114

samples were tested for IgG dengue, of these 34 samples were positive.

To implement public health control programmes, it is nice to understand the male female difference in dengue infection rate and the complications of the disease. In this study higher prevalence of dengue infection was noted among males (57.36%) than females (42.64%) with a male: female ratio being1.3:1 seen in this study. The most affected age group was 20-29 years followed by 30-39 years as 34.4% and 32.5%. In another study by Hari. P Nepal et al the highest number of dengue cases was observed in the 21-30 years age group with greater predilection in males than in females. In this study 62% of positive cases were males (31/50) and 38% were females (19/50) with a, a male to female ratio of 1.6:1.[17]

In a study conducted in Rewa district of Madhya Pradesh, a total of 1113 sample tested for dengue the prevalence of dengue was higher in male (12.94%) in comparison to females (5.54%).[18] A male: female ratio being1.3:1. A male: female ratio of 2:1 seen in a study by Atul Garg et al.[19] In another study, females (57.5%) were more affected more than the males with a male to female ratio of 1:1.35.[19]

In this study the most affected age group was 20-29 years followed by 30-39 years as 34.4% and 32.5%. In a study by Manisha et al,[20] 21-30 years age group was most affected. In a study by Prabhakar et al,[5] the most affected age group was 15 to 30 years, with 13 (31.71%), followed by the 5--9-year age group, with 08 (19.51%) and the male-to female ratio was found to be 2.15:1.

The correlation between occurrence of dengue and monsoon season is clearly evident in this study and is further supported by similar findings from Delhi, Ludhiana, Lal et al,[21] 2007; and from Chandigarh by Ratho et al 2005.[22] In another study by Amitkumar et al, most of the samples 161 (82.14%) were received during monsoon and post monsoon period (June- November) with high positivity 27 (62.79%) for Dengue during post monsoon period (October- November).[23]

Prevention or reduction of dengue virus transmission depends mainly on control of the mosquito vectors or interruption of human-vector contact. Reduction of mosquito habitat to eliminate pockets of stagnant water that serve as mosquito breeding sites at home, workplaces and their vicinity, are the best way to prevent dengue fever stay in well-screened housing, protective clothing, usage of mosquito repellent may also reduce the dengue**.** Community participation, regular epidemiological surveillance, integrated vector control measures active participation of Government and non-government organizations for initiation of preventive strategies can reduce the rate of dengue.

Conclusion

According to the present study, dengue fever mainly seen in active adult male population. Increase in prevalence of dengue during monsoon and post monsoon season may be due to the abundant number of vectors and rapid urbanization. NS1 in combination with IgM assay offers the most sensitive and cost-effective diagnostic method for acute dengue infection.

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